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Inoculation of *Acacia mangium* with Alginate Beads Containing Selected *Bradyrhizobium* Strains under Field Conditions: Long-Term Effect on Plant Growth and Persistence of the Introduced Strains in Soil

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The growth response of *Acacia mangium* Willd. to inoculation with selected *Bradyrhizobium* strains was investigated in two field trials in the Ivory Coast (West Africa). In the first trial (Anguededou), four provenances (i.e., trees originating from seeds harvested in different geographical areas) of *A. mangium* were inoculated with four *Bradyrhizobium* strains from different origins. Six months after being transplanted in the field, the heights of all inoculated trees showed a statistically significant increase of 9 to 26% compared with those of uninoculated trees, with the most effective strain being Aust 13c. After 19 months, the positive effect of inoculation on tree growth was confirmed. The effect of *A. mangium* provenance on tree growth was also highly significant. Trees from the Oriomo provenance of Papua New Guinea had a mean height that was 25% greater than those of other provenances. Analysis of variance showed a highly significant effect of interaction between strain and host provenance factors. Thus, most effective strain × provenance combinations could be proposed. Immunological identification of strains clearly showed that 90 to 100% of nodules from trees inoculated with three of the four *Bradyrhizobium* strains or from uninoculated trees contained exclusively Aust 13c 23 months after tree transplantation. This predominance of Aust 13c in nodules was still observed 42 months after tree transplantation. The second experiment (Port-Bouët), performed with a different soil, confirmed the long-term positive effect of Aust 13c on plant growth, its high competitive ability against indigenous strains, and its persistence in soil. Strain Aust 13c should thus be of great interest for inoculating *A. mangium* under a wide range of field conditions.

Acacia mangium is a legume tree of the humid tropics renowned for its very fast growth, even in depleted soils. *A. mangium* is often spontaneously nodulated by rhizobia in its native area and in soils where it has been introduced. To achieve maximal growth when introduced in N-deficient soils, it is recommended that *A. mangium* be inoculated with specific and effective strains of rhizobium, although it nodulates readily with indigenous strains of rhizobium (10). Short-term nursery experiments performed by Umali-García et al. (17) showed that the inoculation of *A. mangium* with certain strains of rhizobium had a positive effect on tree biomass (expressed as total N) in nonsterile soils (Annam clay soils in the Philippines), but the authors did not specify if the effective strains belonged to the *Rhizobium* group or the *Bradyrhizobium* group. In 1990, Galiana et al. (8) showed that only a restricted range of *Bradyrhizobium* strains were able to produce effective nodules on *A. mangium* and that *A. mangium* was a specific host since the effectiveness of these different strains varied considerably. However, these results were obtained under laboratory conditions from plants grown on a synthetic substrate supplemented with a N-free nutrient medium. In fact, very few experiments have been undertaken to evaluate the effectiveness of introduced strains and to assess their persistence in nodules for several years in the field.

This paper aimed at estimating the effect of inoculation on *A. mangium* growth under field conditions with *Bradyrhizobium* strains previously selected on the basis of their effectiveness as evaluated under laboratory conditions (8). Field inoculation trials were established at two sites in the southern Ivory Coast to determine (i) the long-term effects of different *Bradyrhizobium* strains on the growth of *A. mangium*, (ii) the effects of different *A. mangium* provenances on tree growth and their interactions with the *Bradyrhizobium* strains used, and (iii) the occurrence of introduced strains in nodules several years after inoculation and their competitiveness against indigenous strains.

MATERIALS AND METHODS

Sites and soils. (i) Experiment 1. This experiment was conducted from 1988 to 1991 at the Anguededou station (5°25′N, 4°10′W) of the Institut des Forêts-Département de la Foresterie (IDEFOR/DFO) on a tertiary sandy clay soil. The soil had a pH (H_2O) of 4.22, total N content of 880 μg g of dry soil^{-1}, P content of 51 μg g of dry soil^{-1}, K content of 0.06

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TABLE 1. Origins of *Bradyrhizobium* strains used in experiments

Strain	Host plant	Source	Reference
Aust 1b	*A. mangium*	Australia	This study
Aust 11c	*A. mangium*	Australia	This study
Aust 13c	*A. mangium*	Australia	8
PBG3	*A. mangium*	Ivory Coast	8
AG3	*A. mangium*	Ivory Coast	8
RMBY	*A. mangium*	Senegal	6
ORS 800	*A. holosericea*	Senegal	5
TAL 72	*Albizzia falcataria*	Mexico	9
CB 756	*Macrotyloma africanum*	Zimbabwe	9

meq/100 g of dry soil, and organic C content of 1.36%. The experiment site had initially been the site for the planting of a non-nitrogen-fixing tree, *Cordia alliodora* (*Boraginaceae*), and had recently been cleared.

(ii) Experiment 2. This experiment was conducted from 1990 to 1991 at Port-Bouët (5°15′N, 4°10′W) on a quaternary coastal sandy soil. The soil pH (H_2O) ranged from 4.2 to 4.9; there was a total N content of 410 to 1,330 μg g of dry soil^{-1}, P content of 88 to 210 μg g of dry soil^{-1}, K content of 0.03 to 0.10 meq/100 g of dry soil, and organic C content of 0.42 to 1.40%. Before planting, the vegetation of this site was characterized by two dominant species, *Imperata cylindrica*, which is a representative gramineous weed of degraded soils of low pH, and *Pueraria phaseoloides*, a perennial legume cover crop spontaneously nodulated by indigenous strains.

Plant material. (i) Experiment 1. Seeds of the following four *A. mangium* provenances (trees originating from seeds harvested in a well-defined geographical area), supplied by the Centre de Coopération Internationale en Recherche Agronomique pour le Développement-Département Forêt, were used in this experiment: Piru Ceram, Indonesia (seedlot number 86/6666 N); Rex Range, North Queensland, Australia (seedlot number 83/4136 N); Oriomo, Papua New Guinea (seedlot number 84/4609 N); San Pedro, Ivory Coast (IDEFOR/DFO seedlot).

(ii) Experiment 2. All *A. mangium* seeds originated from the San Pedro, Ivory Coast (IDEFOR/DFO seedlot) provenance. The *Eucalyptus urophylla* trees forming the intercalary rows of the experimental design between *A. mangium* plots (see below) originated from a local provenance (IDEFOR/DFO seedlot).

***Bradyrhizobium* strains.** The *Bradyrhizobium* strains (slowly growing rhizobia belonging to the cowpea miscellany group) used in this study are listed in Table 1.

(i) Experiment 1. Four *Bradyrhizobium* strains were tested, Aust 13c, AG3, RMBY, and TAL 72. Aust 13c had previously been selected for its superior effectiveness when *A. mangium* seedlings grown on N-free nutrient medium were inoculated with it (8). Under similar growing conditions, RMBY, AG3, and TAL 72 were shown to be less effective than Aust 13c. AG3 was selected because it is a strain indigenous to Anguededou and isolated from *A. mangium* nodules collected close to the site of the field experiment. RMBY was chosen because it, as well as AG3, is an African strain isolated from *A. mangium* nodules.

(ii) Experiment 2. Two *Bradyrhizobium* strains were tested, Aust 13c and CB 756. Compared with Aust 13c, CB 756 was shown not to be very effective when *A. mangium* seedlings were inoculated with it (8).

Plant inoculation. (i) Experiment 1. *A. mangium* seeds were pretreated by immersion in boiling water, soaked for one night, and germinated on sterilized sand. After 1 week, young seedlings were placed in 1-liter polyethylene bags filled with soil from the planting site and were inoculated with the four *Bradyrhizobium* strains. This soil had been previously disinfected with 0.3 g of Maposol (Procida Co., Marseille, France) per kg of soil, i.e., 0.144 g of metam sodium per kg as the active ingredient. For uninoculated controls, half of the plants were transferred to bags containing disinfected soil and half to bags with nondisinfected soil. Inoculants were prepared by entrapping 7-day-old pure cultures of each of the four *Bradyrhizobium* strains, previously grown on a yeast mannitol medium (18) containing 10^9 cells per ml, in a 5% alginate pseudosolution according to the usual procedure (4). Before being applied to seedlings, air-dried beads of inoculant were rehydrated in a 0.1 M phosphate buffer, pH 7.4, for 10 h with 5 g of inoculant per liter of buffer. After homogenization, inoculant was applied to plants at a rate of 16 ml per container, corresponding to 10^8 bacteria per plant. This same polymeric inoculant rehydrated in phosphate buffer but without entrapped bacteria was applied to uninoculated control plants. Plants were grown in the nursery and placed under shade on a layer of previously sterilized coarse gravel to ensure good drainage and to limit cross-contamination between treatments or with indigenous strains. After 2 months of growth (1 April to 30 May 1988), plants were transplanted to the field.

(ii) Experiment 2. Seed germination and plant inoculation were carried out as described above except that 10% kaolinite was added to 5% alginate before its polymerization (15 g of inoculant per liter of 0.1 M phosphate buffer was then used for rehydration). All seedlings were grown on autoclaved soil (120°C for 1 h) from the planting site. Plants were transferred to the field after 2 months of growth in the nursery (15 March to 15 May 1990).

Experimental designs. (i) Experiment 1. For each *A. mangium* provenance, six *Bradyrhizobium* strain treatments were tested, giving a total of 24 strain × provenance combinations. These combinations were replicated three times in a split plot design of 1.012 ha. Each of the three blocks contained five subblocks, with four of these subblocks corresponding to one *A. mangium* provenance and one to a provenance of *A. auriculiformis* (the last one is not considered in this study). Each of the subblocks contained six *Bradyrhizobium* strain treatments. Plots were separated by one row of *A. mangium* (uninoculated trees of the San Pedro provenance) to avoid cross-contaminations between plots. Tree spacing was 2.5 by 2.5 m, with 10 (two rows of 5 trees) *A. mangium* trees per plot.

(ii) Experiment 2. The field trial consisted of three treatments replicated in three blocks of a randomized complete block design of 0.783 ha. Each block contained three plots. One plot had 30 trees inoculated with strain Aust 13c; another had 30 trees inoculated with strain CB 756; the last one had 30 uninoculated trees (control). *A. mangium* plots were separated by three to six rows of *E. urophylla* to prevent cross-contamination between plots. Tree spacing was 3 by 3 m, with 30 (six rows of 5 trees) *A. mangium* trees per plot. The cover vegetation of blocks I and II had originally been *P. phaseoloides*, and that of block III had been *I. cylindrica*.

Data analysis. (i) Experiment 1. Growth in height was recorded every 2 months for 6 months after field transplantation. Nineteen months after field transplantation, tree height and basal area at 1.3 m (basal area = $C^2/4\pi$ where C is the circumference at a height of 1.3 m or $\Sigma C_i^2/4\pi$ when trees were multicauline) were analyzed. Data were subjected to a three-way analysis of variance by using the Statistical Analysis System computer program, and means were compared with the Newman and Keuls Multiple Range Test (13).

(ii) Experiment 2. Nineteen months after field transplantation, tree height, circumference, and basal area at ground level

TABLE 2. Effects of the *Bradyrhizobium* strain and *A. mangium* provenance factors on tree height during 6 months of field growth in experiment 1 at Anguededou

Factor[a]	Height (cm tree^{-1})[b]		
	2 mo	4 mo	6 mo
Strain			
Aust 13c	45.6 b (+99%)	105.6 a (+54%)	215.7 a (+26%)
AG3	41.6 c (+82%)	93.4 b (+36%)	198.0 b (+16%)
RMBY	47.1 b (+106%)	98.7 ab (+44%)	201.9 b (+18%)
TAL 72	51.3 a (+124%)	105.2 a (+53%)	210.2 ab (+23%)
Uninoculated D	29.4 d (+28%)	80.9 c (+18%)	187.0 c (+9%)
Uninoculated ND	22.9 e	68.7 d	171.0 d
Provenance			
Piru Ceram	49.0 a	112.5 a	222.9 a
Rex Range	38.9 b	84.7 bc	189.4 c
Oriomo	35.8 c	93.0 bc	206.9 b
San Pedro	34.5 c	79.4 c	184.5 c

[a] Strain factor is for all provenances combined; the provenance factor is for all strains combined. Uninoculated D, uninoculated control plants initially grown at the nursery on soil disinfected with metam sodium. Uninoculated ND, uninoculated control plants initially grown on nondisinfected soil. Plants inoculated with the four *Bradyrhizobium* strains were grown on soil initially disinfected with metam sodium.

[b] Values (means of 120 replicates for strain effect and 180 replicates for provenance effect) followed by the same letter are not significantly different according to the Newman and Keuls test ($P = 0.05$). Percentage difference compared with that of uninoculated trees grown on nondisinfected soil (uninoculated ND) appears in parentheses.

TABLE 3. Effects of the *Bradyrhizobium* strain and *A. mangium* provenance factors on tree height and basal area after 19 months of field growth in experiment 1 at Anguededou[a]

Factor[b]	Height (m tree^{-1})	Basal area at 1.3 m (cm^2 tree^{-1})
Strain		
Aust 13c	11.3 a (+3.7%)	90.0 a (+23.6%)
TAL 72	11.2 a (+2.7%)	89.3 a (+22.7%)
AG3	10.4 a (−4.6%)	83.9 a (+15.2%)
RMBY	11.8 a (+8.3%)	81.3 a (+11.7%)
Uninoculated D	10.8 a (−0.9%)	82.2 a (+12.9%)
Uninoculated ND	10.9 a	72.8 b
Provenance		
Oriomo	13.0 a	88.2 a
San Pedro	10.8 b	86.6 a
Rex Range	10.3 b	74.3 a
Piru Ceram	10.2 b	84.2 a

[a] See Table 2, footnote *b*. Differences were not significant with the strain factor for height nor with the provenance factor and the interaction between the strain and provenance factors for basal area. The interaction between the strain and provenance factors was significant ($P < 0.05$) for height. The strain factor and the provenance factor were very significant ($P < 0.01$) for basal area and height, respectively.

[b] See Table 2, footnote *a*.

were analyzed. Data were subjected to a two-way analysis of variance, and means were compared with the Newman and Keuls Multiple Range Test.

Serological analyses and serological relatedness among *Bradyrhizobium* strains. This experiment was intended to identify possible cross-reactions between the strains used for inoculating *A. mangium* and between these strains and others of different origins (Table 1). Rabbit antisera were prepared from strains Aust 13c, Aust 11c, Aust 1b, PBG3, AG3, RMBY, ORS 800, TAL 72, and CB 756. The indirect fluorescein isothiocyanate-labelled antibody technique was used with serial dilutions of antisera against these strains from 1:10 to 1:5,120 (14). Homologous and heterologous reactions were assessed by estimation of the degree of fluorescence from − (no fluorescence) to ++++ (bright fluorescence). Serological relatedness among strains was determined according to the dilution of antiserum giving maximal fluorescence.

Nodule occupancy in field experiments and nodule sampling. (i) Experiment 1. For serotyping, fresh nodules were collected in the field trial 23 and 42 months after field transplantation, April 1990 and November 1991, respectively. Nodule sampling in April 1990 was carried out in one subblock of the San Pedro provenance in block I. Nodule sampling in November 1991 was carried out in two subblocks of the Rex Range provenance located in blocks I and II. One tree per plot was sampled. Samples of 50 nodules per tree were taken at random within a 1-m radius around the trunk base from the soil surface to a depth of 15 cm. Nodules were washed with tap water before dehydration and storage in sealed tubes containing silica gel.

(ii) Experiment 2. Fresh nodules were collected in the field trial 19 months after field transplantation (December 1991). Root nodules were collected on two trees per plot, with these trees located at the same places in all plots. Nodules were sampled and conditioned as described for experiment 1.

Serological typing of bacteroids. (i) Experiment 1. The identification of rhizobium strains in nodules was done by the indirect fluorescein isothiocyanate-labelled antibody technique (14). Ten nodules randomly taken from the 50 sampled per tree were analyzed. Nodules were crushed and steamed in physiological saline at 100°C for 1 h, and bacteroids were purified by centrifugation. Bacteroids were tested as antigens against antisera of the four strains used in the field experiment, Aust 13c, AG3, RMBY, and TAL 72.

(ii) Experiment 2. Nodules were characterized according to the procedure described above. Bacteroids from 10 nodules per tree, two trees per plot, and nine plots (three plots per block and three blocks) were tested as antigens against antisera of the two strains used in the field experiment, Aust 13c and CB 756, and against PBG3 antiserum used as a control, since PBG3 is an indigenous strain from Port-Bouët isolated from *A. mangium* nodules collected close to the site of the field experiment.

RESULTS

Effects of inoculation with *Bradyrhizobium* strains on tree growth. (i) Experiment 1. (a) Inoculation effects during the first 6 months of field growth. Statistical analysis of data showed significant effects for the three factors tested, *Bradyrhizobium* strain, *A. mangium* provenance, and block. Six months after field transplantation, trees inoculated with the four *Bradyrhizobium* strains, all provenances combined, had a total height that was 9 to 26% significantly greater ($P = 0.05$) than that of uninoculated control trees grown previously in the nursery on disinfected soil or nondisinfected soil (Table 2). Trees inoculated with Aust 13c had a height that was 3 to 9% significantly greater ($P = 0.05$) than that of trees inoculated with the other three strains, although TAL 72 was the most effective 2 months after transplantation. The differences between treatments decreased with time, for they were 40 to 125% according to the strain used after 2 months of growth. Furthermore, uninoculated control plants grew better on disinfected soil than on nondisinfected soil.

As shown in Table 2, the effect of provenance on tree height

TABLE 4. Effects of the *Bradyrhizobium* strain and block factors on height, circumference, and basal area of *A. mangium* trees after 19 months of field growth in experiment 2 at Port-Bouët[a]

Factor[b]	Height (m tree^{-1})	Circumference at ground level (cm tree^{-1})	Basal area at ground level (cm^2 tree^{-1})
Strain			
Aust 13c	11.1 a (+15.0%)	59.2 a (+10.2%)	282 a (+20.0%)
CB 756	9.6 c (−1.0%)	53.4 c (−0.6%)	231 c (−1.7%)
Uninoculated	9.7 b	53.7 b	235 b
Block			
I	10.0 b	59.3 a	284 a
II	11.0 a	55.6 b	248 b
III	9.3 c	51.2 c	213 c

[a] Values (means for 90 replicates) followed by a different letter are significantly different according to the Newman and Keuls test ($P = 0.01$). Percentage difference compared with that of uninoculated control trees appears in parentheses. The interaction between the strain and block factors was not significant for height, circumference, or basal area. However, each factor individually was significant ($P < 0.001$) for all three parameters tested.

[b] Strain factor is for all blocks combined; block factor is for all strains combined.

was also significant. Thus, trees of the Piru Ceram provenance had a total height that was significantly greater ($P = 0.05$) than trees of the other three provenances during the first 6 months of growth for all *Bradyrhizobium* strain treatments combined.

(b) Inoculation effects after 19 months of field growth. Table 3 shows that the positive effect of inoculation on the growth in height for all *A. mangium* provenances combined was maintained after 19 months of growth, although the differences between trees inoculated with the four *Bradyrhizobium* strains and uninoculated control trees were not significant at $P = 0.05$. In contrast, the effect of the *Bradyrhizobium* strain factor on the basal areas of trees was highly significant ($P = 0.003$). The basal area of trees inoculated with the four *Bradyrhizobium* strains and that of uninoculated control trees initially grown on disinfected soil in the nursery (uninoculated D) were significantly higher than that of uninoculated trees grown initially on nondisinfected soil in the nursery (uninoculated ND), about 25% higher with strain Aust 13c, which was the most effective.

Contrary to the *Bradyrhizobium* strain factor effect, the effect of *A. mangium* provenance on height for all *Bradyrhizobium* strain treatments combined was highly significant at $P = 0.003$ after 19 months of growth. Thus, trees of the Oriomo provenance had a mean height of 13.0 m, about 25% higher than those of the other three provenances (Table 3). The basal area of Oriomo provenance trees was also greater, although no significant differences from the other provenances were shown at $P = 0.05$.

Variance analysis also showed a highly significant effect for the interaction between the *Bradyrhizobium* strain factor and the *A. mangium* provenance factor on tree height ($P = 0.01$). Thus, some strain × provenance combinations, such as AG3 × San Pedro, RMBY × Rex Range, and RMBY × Piru Ceram, had a synergical effect on height which was comparable to that of combinations including the Oriomo provenance (results not shown). On the contrary, some combinations had a negative effect; for example, trees of the Oriomo provenance inoculated with AG3 were much shorter (10.2 m) than those from the same provenance inoculated with the other three strains (Aust 13c, 13.7 m; TAL 72, 14.7 m; RMBY, 12.6 m) or not inoculated (uninoculated D, 13.8 m; uninoculated ND, 13.1 m). In contrast, there was no significant interaction between the two factors for basal area ($P = 0.05$). The most efficient strain × provenance combination was that of trees of the Oriomo provenance inoculated with TAL 72, with a height that was 5 to 57% greater than those of the other combinations and a basal area that was 2 to 86% greater (results not shown).

(ii) Experiment 2. As indicated in Table 4, the results obtained at Port-Bouët showed a positive and highly significant effect ($P < 0.001$) of inoculation with strain Aust 13c on tree growth 19 months after field transplantation for all blocks combined. Thus, the height of trees inoculated with Aust 13c was 15% greater than that of trees inoculated with CB 756 and that of uninoculated ones. Likewise, the circumference and basal area of trees inoculated with Aust 13c were 10 and 20% larger, respectively, than those measured in other strain treatments. The block effect on tree growth was also highly significant ($P < 0.001$) for all strain treatments combined. Indeed, tree height was greater in block II than in blocks I and III, 10 and 18.3% greater, respectively, whereas tree circumference and basal area were greater in block I than in blocks II and III, with a difference of 14.5 and 33.3%, respectively, for basal area. Furthermore, there was no effect from the interaction of the strain and block factors on measured parameters. This absence of interaction means that the treatment in the plot inoculated with Aust 13c was the most efficient in each of the three blocks and that the ranking of blocks was not affected by strain treatment (detailed results not reported).

Serological relatedness among *Bradyrhizobium* strains. Rabbit antisera raised against the nine strains studied exhibited immunofluorescence titers of 1:320 to 1:640 (Table 5). The cross-reactions described in Table 5 occurred principally be-

TABLE 5. Serological relatedness of nine *Bradyrhizobium* strains[a] by immunofluorescence reactions

Strain	Immunofluorescence reaction with antiserum[b]								
	Aust 13c	Aust 11c	Aust 1b	PBG3	AG3	TAL 72	RMBY	ORS 800	CB 756
Aust 13c	+++	−	+	−	−	−	−	−	−
Aust 11c	+	+++	+++	−	−	−	−	−	−
Aust 1b	−	++	++++	−	−	−	−	−	−
PBG3	−	−	−	+++	++	+	−	−	−
AG3	−	−	−	+	+++	ND	−	−	−
TAL 72	−	−	−	−	−	+++	ND	++	−
RMBY	−	−	−	−	ND	+++	++++	+++	−
ORS 800	−	−	−	+	+	−	−	++++	−
CB 756	−	−	−	−	−	−	−	−	+++

[a] See Table 1 for strain origins.

[b] ++++, positive reaction with antiserum at a dilution of 1:640 or more; +++, positive reaction with antiserum at a dilution of 1:320 or 1:160; ++, positive reaction at a dilution of 1:80 or 1:40; +, positive reaction at a dilution of 1:20 or 1:10; −, no cross-reaction. ND, not determined.

TABLE 6. Nodule occupancy of inoculated and uninoculated trees in field inoculation trial at Anguededou (experiment 1)

Mo after tree transplantation	Block	Tree provenance	Inoculated strain[a]	No. of nodules reacting positively with antiserum[b]			
				Aust 13c	RMBY	TAL 72	AG3
23	I	San Pedro	Aust 13c	10	0	0	0
			RMBY	6	4	5	0
			TAL 72	9	0	0	0
			AG3	10	0	0	0
			Uninoculated D	9	0	0	0
			Uninoculated ND	9	0	0	0
42	I	Rex Range	Aust 13c	10	ND	ND	ND
			RMBY	10	2	2	0
			TAL 72	10	1	0	2
			AG3	10	0	1	0
			Uninoculated D	ND	ND	ND	ND
			Uninoculated ND	ND	ND	ND	ND
42	II	Rex Range	Aust 13c	10	1	0	0
			RMBY	10	0	0	0
			TAL 72	7	0	0	0
			AG3	10	0	0	0
			Uninoculated D	9	0	0	0
			Uninoculated ND	7	0	0	0

[a] Uninoculated D, uninoculated control plants initially grown at the nursery on soil disinfected with metam sodium. Uninoculated ND, uninoculated control plants initially grown on nondisinfected soil. Plants inoculated with the four *Bradyrhizobium* strains were grown on soil initially disinfected with metam sodium.

[b] Nodules were analyzed by the indirect immunofluorescence technique. Ten nodules per tree were tested, and each nodule was analyzed with each of the four antisera. ND, not determined.

tween strains of the same geographical origin. Thus, four serogroups defined by the resulting immunofluorescence heterologous reactions were identified. The first serogroup included three Australian strains, Aust 1b, Aust 11c, and Aust 13c; the second serogroup had two *A. mangium* strains, PBG3 and AG3, originating from the Ivory Coast; the third serogroup included three strains, TAL 72 from Mexico and RMBY and ORS 800 from Senegal; and the fourth serogroup was represented by strain CB 756, considered to be a reference strain for *Bradyrhizobium* spp. belonging to the cowpea group (9). However, strain ORS 800 from the third serogroup cross-reacted with antisera from strains PBG3 and AG3 of the second serogroup. This experiment also showed that the *Bradyrhizobium* strains used in each of the field experiments belonged to different serogroups and could be easily distinguished, except for RMBY, which cross-reacted with TAL 72, both used in field experiment 1.

Identification of *Bradyrhizobium* strains in nodules collected in field experiments. (i) Experiment 1. As shown in Table 6, immunofluorescence identification of the bacteroids in root nodules collected at Anguededou clearly showed the predominance of strain Aust 13c both 23 and 42 months after field transplantation, whatever the block or provenance (subblock) studied. All nodules collected on trees inoculated with Aust 13c contained Aust 13c exclusively. Furthermore, 100% of the bacteroids observed under the UV microscope in all nodules reacting exclusively with Aust 13c antiserum were fluorescent and identified as Aust 13c (not specified in Table 6). In the same way, most of the root nodules collected from trees inoculated with RMBY, TAL 72, and AG3 and from uninoculated trees (uninoculated D and uninoculated ND) contained Aust 13c, whatever the time after tree transplantation, block number, and provenance subblock analyzed, with a total mean of 8.9 of 10 nodules (Table 6). Similarly, double-strain occupancy was found in few nodules and 100% of the bacteroids in nodules reacting positively and exclusively with Aust 13c antiserum were fluorescent. Among the other three introduced strains, only RMBY was recovered at a significant rate, with 40% of nodules containing the RMBY inoculant strain exclusively 23 months after field transplantation (Table 6). Note that these nodules reacted positively with both RMBY and TAL 72 antisera by virtue of the cross-reaction between TAL 72 antiserum and strain RMBY (Table 5).

(ii) Experiment 2. As clearly indicated in Table 7, immunofluorescence identification of the bacteroids in root nodules collected at Port-Bouët showed the persistence of Aust 13c 19 months after field transplantation, whatever the block or tree

TABLE 7. Nodule occupancy of inoculated and uninoculated trees in field inoculation trial at Port-Bouët (experiment 2) after 19 months of field growth

Inoculated strain	Block	Tree no.	No. of nodules reacting positively with antiserum[a]		
			Aust 13c	CB 756	PBG3
Aust 13c	I	1	10	0	0
		2	10	0	0
	II	1	10	0	0
		2	10	0	0
	III	1	10	0	0
		2	ND	0	0
CB 756	I	1	0	1	1
		2	0	0	0
	II	1	0	0	0
		2	2	0	0
	III	1	2	0	0
		2	9	0	0
Uninoculated	I	1	0	0	0
		2	1	0	0
	II	1	1	2	0
		2	0	2	0
	III	1	6	0	0
		2	2	0	ND

[a] Nodules were analyzed by the indirect immunofluorescence technique. Ten nodules per tree were tested, and each nodule was analyzed with each of the three antisera. ND, not determined.

studied. Thus, all nodules collected from trees inoculated with Aust 13c reacted with homologous antiserum. Furthermore, 100% of the bacteroids observed were fluorescent and identified as Aust 13c (not specified in Table 7). In contrast, CB 756 was not recovered in nodules from trees inoculated with this strain. However, CB 756 was found in a few nodules from two uninoculated trees located in block II. On the other hand, Aust 13c contaminated two plots of block III planted with trees inoculated with CB 756 or not inoculated. Hence, two trees showed a majority of root nodules containing Aust 13c, while two other trees had a few nodules containing both Aust 13c and indigenous strains. All other nodules collected on trees inoculated with CB 756 or not inoculated contained a majority of indigenous strains that did not react with any of the antisera tested. Surprisingly, very few nodules containing indigenous strains cross-reacted with PBG3 antiserum, although strain PBG3 originated from Port-Bouët and thus might be serologically related to many indigenous strains.

DISCUSSION

Our study clearly demonstrates that *A. mangium* responds positively to inoculation with *Bradyrhizobium* strains under field conditions. This is the first published work to show a positive effect of inoculation on the growth of a leguminous tree species maintained beyond 1 year under sylvicultural conditions. Other field studies reported by Norris (11) and Sanginga et al. (12) have demonstrated that *Leucaena leucocephala* also can respond to inoculation with specific *Rhizobium* strains, but their experiments did not exceed 6 months. The effect of inoculation on the field growth of tree species nodulated exclusively with *Bradyrhizobium* strains, even at an early stage of growth, has not previously been reported.

In addition to data previously obtained in vitro (8), the results presented in this paper show that an Australian strain, Aust 13c, from the native area of *A. mangium* (North Queensland) was significantly more effective than strains from the Ivory Coast, AG3 and PBG3, or strains from other host plants, TAL 72 and CB 756. This finding is in accordance with the results from our field inoculation experiments on Cook Islands and in Benin (15).

Our data show a positive effect of inoculation with certain *Bradyrhizobium* strains over uninoculated control trees nodulated by indigenous strains. These findings thus indicate that *A. mangium* can be nodulated by a wide range of strains (introduced or native) but with different levels of symbiotic effectiveness. *A. mangium* is thus promiscuous for nodulation but more specific in terms of effectiveness, with different levels of specificity, depending on the provenance tested as was shown in experiment 1. However, it is generally assumed that species nodulating with a *Bradyrhizobium* strain often do not respond to inoculation, unlike species nodulating with a *Rhizobium* strain, since they are considered to be promiscuous and specific host species, respectively. The data obtained by Turk et al. (16) with different legume tree species grown in four Hawaiian soil types support such a general view. In their pot experiment performed with nonsterile soils, these authors found that only *L. leucocephala*, *Sesbania grandiflora*, and *Robinia pseudoacacia* (nodulated with a *Rhizobium* strain) responded positively and significantly to inoculation, whereas *A. auriculiformis*, *A. mearnsii*, and *A. mangium* (nodulated with a *Bradyrhizobium* strain) did not respond in most cases. The very low levels of *A. mangium* response to inoculation in their study could be due to the short duration of the experiment (9 weeks), given that the initial growth of this species is known to be very slow, or to the poor effectiveness of the strains tested.

Furthermore, we found a significant effect of interaction between the *Bradyrhizobium* strain and tree provenance on tree height, which may suggest some affinities and a certain degree of compatibility in some strain × provenance combinations. We observed that the combination of Oriomo provenance with AG3 produced a negative effect on tree height, even when compared with uninoculated trees. One explanation for this could be the total occupancy of infection sites by a more infective but less effective strain, with the low effectiveness due to the particular AG3 strain × Oriomo provenance combination. This is an important fact to be considered by people interested in field inoculation experiments when choosing strain × provenance combinations.

The contamination and nodulation of all trees by strain Aust 13c in experiment 1 could explain the decrease of the positive effect of inoculation on tree growth and the reduction in differences between treatments with time. In this experiment, the short spacing between plots (delimited by a single border row of uninoculated trees) and between trees (2.5 by 2.5 m) probably favored dissemination of Aust 13c to adjacent plots. Our results contrast with studies showing that bradyrhizobial migration from the initial point of inoculation was very limited. This restricted spread of the inoculum has been shown with various host species, such as *Glycine max* (19) and perennial tropical forage legumes *Desmodium intortum*, *Macroptilium atropurpureum*, and *Neonotonia wightii* (3). In such species, the distribution of nodules containing the introduced strains was restricted to the vicinity of the tap root, while the extending lateral roots were nodulated by indigenous strains. On the contrary, *A. mangium* lateral roots were nodulated by the inoculated strain Aust 13c at distances of up to a 1-m radius around the trunk base. In the second experiment carried out at Port-Bouët, *A. mangium* plots were more distant from each other since three to five rows of intercalary rows of eucalypts separated them and thus limited strain contamination between plots. Thus, the positive effect of inoculation on tree growth was more pronounced at Port-Bouët as trees inoculated with CB 756 and uninoculated control trees were all nodulated by less effective indigenous rhizobia.

The exceptional persistence of introduced strains over several years of plant growth, as indicated here by their presence in nodules, has rarely been demonstrated for perennial herbaceous species and never for tree legumes. In the case of *Rhizobium* strains associated with annual legumes, several studies with *Bradyrhizobium japonicum* have shown that highly competitive strains (e.g., USDA 123 serogroup) occur and persist in the soils of most soybean-growing areas (2, 7). Nodule occupancy by such persistent strains can reach 100%, even after inoculation with other strains (7). A further study of the effect of the relative competitiveness between indigenous strains of *P. phaseoloides* and Aust 13c on the nodulation of *A. mangium* would probably be worthwhile as *P. phaseoloides*, abundantly nodulated at Port-Bouët, are also known to be associated with *Bradyrhizobium* spp. belonging to the cowpea group (1).

Although *A. mangium* is the most widely planted acacia in many tropical countries, the inoculation of this leguminous tree species with its microsymbiont has not yet been put into practice on a large scale. Although *A. mangium* is a promiscuous tree species spontaneously nodulated by indigenous rhizobia in African soils, our data show that the prior inoculation of *A. mangium* in nurseries with selected *Bradyrhizobium* strains, e.g., Aust 13c, is needed to enhance tree growth and wood production under natural conditions.

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